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REVIEW

Plant viral proteins and fibrillarin: the link to complete the infective cycle

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Abstract

The interaction between viruses with the nucleolus is already a well-defined field of study in plant virology. This interaction is not restricted to those viruses that replicate in the nucleus, in fact, RNA viruses that replicate exclusively in the cytoplasm express proteins that localize in the nucleolus. Some positive single stranded RNA viruses from animals and plants have been reported to interact with the main nucleolar protein, Fibrillarin. Among nucleolar proteins, Fibrillarin is an essential protein that has been conserved in sequence and function throughout evolution. Fibrillarin is a methyltransferase protein with more than 100 methylation sites in the pre-ribosomal RNA, involved in multiple cellular processes, including initiation of transcription, oncogenesis, and apoptosis, among others. Recently, it was found that AtFib2 shows a ribonuclease activity. In plant viruses, Fibrillarin is involved in long-distance movement and cell-to-cell movement, being two highly different processes. The mechanism that Fibrillarin performs is still unknown. However, and despite belonging to very different viral families, the majority comply with the following. (1) They are positive single stranded RNA viruses; (2) encode different types of viral proteins that partially localize in the nucleolus; (3) interacts with Fibrillarin exporting it to the cytoplasm; (4) the viral protein-Fibrillarin interaction forms an RNP complex with the viral RNA and; (5) Fibrillarin depletion affects the infective cycle of the virus. Here we review the relationship of those plant viruses with Fibrillarin interaction, with special focus on the molecular processes of the virus to sequester Fibrillarin to complete its infective cycle.

Keywords GAR domain · Long-distance movement · Cell-to-cell movement · Fibrillarin · Nucleolus · Plant viruses

Introduction

Research on the interaction of viruses and the nucleolus started in the early 1990s. Despite that most viral families have in common is their interaction with the components of the nucleolus [1–4], RNA viruses, in particular those that replicate in the cytoplasm arise more attention. The identification of nuclear and nucleolar localization signals (NLS and NoLS) within viral-encoded proteins sequences explain how those viruses are able to interact with the nucleus and nucleolus [5]. The nucleolus is the main domain of the nucleus. In the nucleolus, functions like gene silencing, cell cycle progression, senescence, ribosomal biogenesis, biogenesis of small nucleolar RNAs, proliferation of RNA and many forms of stress response occur. This region can behave as a dynamic or stable region depending on the nature and quantity of its composed molecules [6–10]. The nucleolus is functionally related to Cajal bodies (CBs), a structure with viral interactions [11]. Fibrillarin (Fib), the main nucleolar protein, is present in both the nucleolus and CBs [12]. Fib is an essential protein conserved in sequence and function throughout evolution [12–14] that is is responsible for the 2'-O-methylations of rRNA and histone H2A in eukaryotes [15, 16]. It belongs to the superfamily of the Rossmann-fold S-adenosylmethionine (SAM) methyltransferases (MTases) [17]. SAM proteins are characterized with a conserved SAM-binding motif, the catalytic triad/tetrad [K-D-K-(H)] and seven-stranded β-sheet flanked by α-helices to form an α-β-α structure [18]. In addition, they have a rich site in arginine and glycine residues (GAR domain) and a specific motif

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to bind RNA. The protein structure can be divided into two domains: the N-terminal and the MTase domain. In plants, the N-terminal domain of *Arabidopsis thaliana* Fib (AtFib) is divided with the GAR domain and a spacer region. Several cellular and viral proteins tend to interact with the GAR domain and give the property of Fib to retain in the nucleolus. Rodriguez-Corona et al. [19] find a novel ribonuclease activity within the AtFib2 GAR domain, whilst AtFib1 do not show. Recently, similar studies found out that this novel activity is conserved in the GAR domain of Homo sapiens Fib (HsFib) [20]. In both (AtFib2 and HsFib), the ribonuclease activity is affected by phosphoinositides. These findings were carried out in vitro, further studies are needed to elucidate the nature of this novel ribonuclease activity. Different viruses from different families require Fib to complete their infective cycle. In this review, we aim to identify possible patterns in Fib's functions exclusively in plant viruses, to expand the knowledge of this protein on plant virology and design novel strategies to control these viruses.

Fibrillarin in viral long distance movement

Groundnut Rossette virus – ORF3

The Groundnut Rosette Virus (GRV) is a virus (+) ssRNA belonging to umbraviruses. Umbraviruses have the peculiarity of not coding a coat protein (CP), so their viral particles are unconventional [21, 22]. GRV ORF3 compensates this deficiency by acting like a CP by associating with the major nucleolar protein, Fib (Table 1). ORF3 traffics from the cytoplasm to the nucleus through its R-rich domain generating the fragmentation of the CBs in Cajal Body-like structure (CBLs) (through a process still unknown) (Fig. 1). Thereby promoting the fusion with the nucleolus and thus recruiting Fib. Although it has not been elucidated yet how ORF3 promotes the formation of CBLs and consequently, the fusion with the nucleolus, a well-documented phenomenon [33, 34], it is possible that the ORF3-Fib interaction interferes with host protein–protein interactions or other processes that affect the integrity of CB [23, 24]. Through Far-Western Blot and mutations analysis, ORF3-Fib interaction was discovered in vitro through the L-rich region (and in particular L149) and the GAR domain, respectively. It is suggested that ORF3 and Fib move as a complex [23, 24]. ORF3-Fib complex is exported from the nucleolus to the cytoplasm with the L-rich domain of ORF3, which acts as a nuclear export signal [35]. Fib's location outside the nucleolus is an indicator of some biotic or abiotic stress. Recruitment in vitro of ORF3 with Fib's and the viral RNA produce filamentous RNP particles with structures and properties similar to those formed in vivo [21, 23, 24]. These RNP particles are infectious and with the ability to protect viral RNA against an RNAase treatment. The binding site of Fib with RNA can serve either to interact physically with viral RNA or to allow catalysis of the RNA or assembly of other proteins with viral RNA, which is unreachable with ORF3 alone. Accordingly, the encapsidation of viral RNA with ORF3 and Fib is sufficient for the formation of infectious RNP filaments capable of LDM in the infection of GRV [23, 24]. The requirement of Fib for GRV LDM is the first evidence reported of a plant virus-Fib interaction. However, to strengthen this hypothesis that Fib plays a role in GRV LDM it would be helpful to obtain precise localization data of Fib in the phloem of GRV-infected plants.

Satellite RNA of bamboo mosaic virus – P20

Satellite RNAs (satRNAs) are parasites of other viruses, referred to as helper viruses (HV) for their replication and movement. satRNAs are generally unrelated in sequence to their HV, although, they depend on HV-encoded proteins for replication and encapsidation [36]. The *Bamboo Mosaic Virus* (BaMV)-associated satRNA (satBaMV) has a single a (+) ssRNA genome. satBaMV encodes a single non-structural protein of 20 kDa (P20) [37] not related to satBaMV

Table 1 Plant virus-encoded proteins described to interact with Fibrillarin

Virus name	Genome	Virus-Encoded Protein	Putative Function in Viral Infection	References
Groundnut rosette virus	(+)ssRNA	Open reading frame 3 (ORF3) protein	Systemic movement	[23, 24]
Potato virus A	(+)ssRNA	Nuclear Inclusion protein a (NIa)	Supression of RNA silencing	[25]
Cucumber mosaic virus	(+)ssRNA	2b	Undetermined	[26]
Poa semilaten virus	(+)ssRNA	Triple gene block protein 1 (TGBp1)	Undetermined	[27]
Beet black scorch virus	(+)ssRNA	P7a	Relevant for viral infection	[28]
Rice stripe virus	(−)ssRNA	P2	Supression of RNA silencing/Systemic movement	[29]
Bamboo mosaic potexvirus satRNA	(+)ssRNA	P20	Systemic movement	[30]
Barley stripe mosaic virus	(+)ssRNA	Triple gene block protein 1 (TGBp1)	Cell-to-cell and systemic movement	[31]
Mulberry mosaic dwarf-associated virus	ssDNA	V2	Supression of RNA silencing	[32]

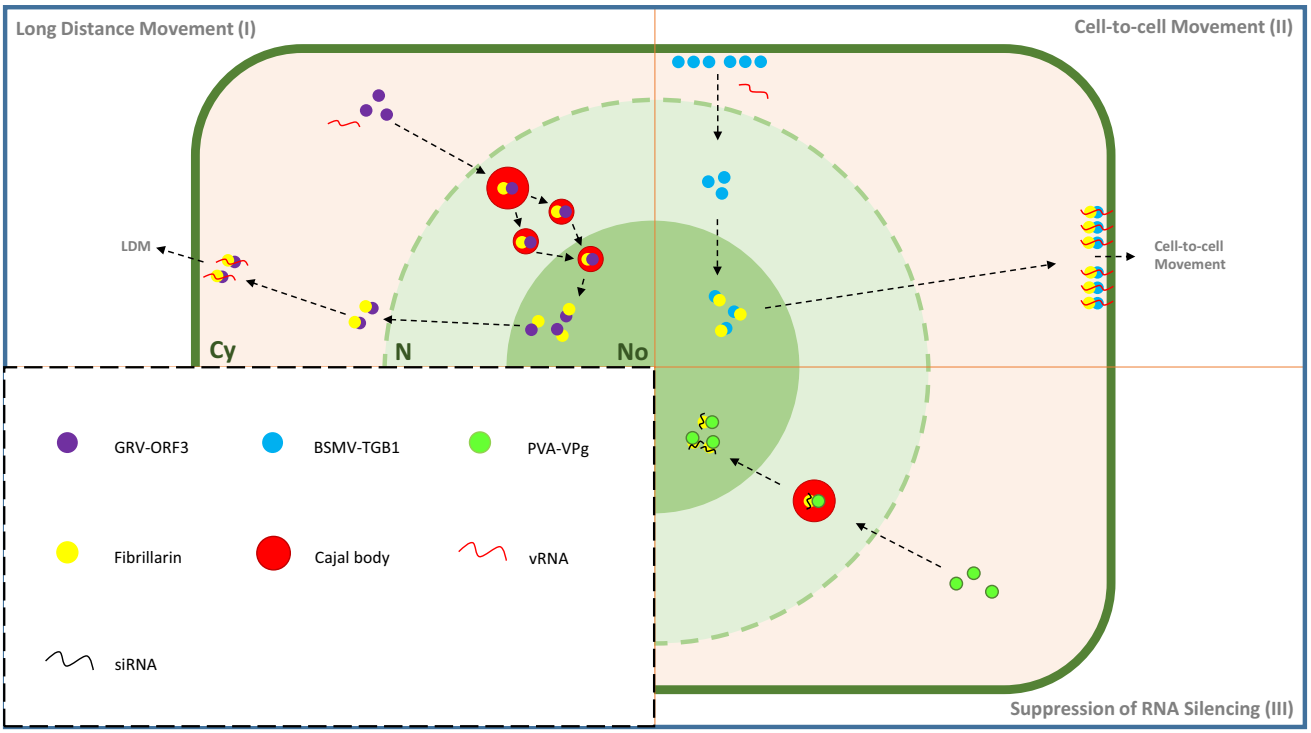

Fig. 1 Model for the role of Fibrillarin in plant viruses. Fibrillarin (Fib) is potentially involved in long distance movement (LDM), cell-to-cell movement and in the suppression of RNA silencing (SRS) of certain single-stranded positive-sense RNA plant viruses. (I) The ORF3 viral protein (VP) of *Groundnut rosette virus* (GRV), mediate its long distance movement (LDM) in part through the interaction with Fib in nucleolus (Nu), Cajal bodies (CBs) (in red circles), and finally in the cytosol (Cy) to form a viral ribonucleoprotein complex (vRNP) to traverse into the phloem. (II) The cell-to-cell movement of *Barley stripe mosaic virus* (BSMV) require the interaction of TGB1 protein and Fib. The TGB1-Fib protein complex localizes in the Nu where are exported to the periphery of the cell to form a vRNP complex where it conducts cell-to-cell movement with other BSMV VPs. (III) A potential, un-described role of Fib in plant viruses might be the SRS. The viral suppressor of RNA silencing (VSR) VPg of *Potato virus A*, interacts with Fib. Fib is an RNA-binding protein in CBs, site of siRNA in plants, which regulates viral defense. The VPg-Fib interaction localizes in the N and Nu, not seen in the Cy. Dotted arrows indicate movement

replication [38]. In fact, BaMV is required for satBaMV replication and encapsidation [37, 39].

The satBaMV P20 localizes in the nucleus and at the cell periphery, where it forms punctate structures [30]. P20 protein inmunoprecipitates with Fib, among other host proteins with anti-P20 IgG through a 7 dpi systemic leaves coinfected with HV and satBaMV, and in the absence of HV. Thus, the role of Fib in satBaMV trafficking (in the absence of HV) was examined by VIGS Fib-silenced *N. benthamiana* plants. The satBaMV RNA was not detected in Fib-silenced scion plants after grafting onto the satBaMV transgenic line, 9 days after grafting (DAG). Quantitative analysis revealed that satBaMV mRNA was greatly reduced in *Fib* RNAi scions. This evidence suggest that Fib is crucial for satBaMV LDM as in GRV infection [23, 24]. However, the effect of fib-silencing impaired only the LDM of satBaMV in HV and satBaMV coinfected plants.

P20-Fib direct interaction was confirmed by yeast two-hybrid (Y2H) assays (Table 1), being consistent with co-IP results [30]. P20 binds to satBaMV and BaMV RNAs with a strong affinity through its arginine-rich motif [40]. In regard to P20 RNA binding property and its interaction with Fib, a potential RNP was studied. RNA extraction from total sap of plants coinfected with HV and satBaMV were incubated with anti-P20 or anti-Fib IgG. RNA from both, HV and satBaMV were present in the total sap incubated with anti-P20 or antiFib IgG. These results indicate that satBaMV P20 with Fib can form RNP complexes in vivo [30], as in GRV infection [23, 24]. In addition, P20 can form punctate structures localized at PD and the satBaMV-P20 RNP complexes can traffic autonomously through the phloem in satBaMV-transgenic stocks or scions [30].

Fibrillarin in viral cell–to–cell movement

Barley mosaic virus – TGB1

Another virus with the genome type (+) ssRNA that requires Fib, is the Barley stripe Mosaic Virus (BSMV), a member

of the genus Hordeivirus (Table 1). The BSMV genome is segmented into three gRNAs designated as α, β and γ. The BSMV movement is orchestrated by CP and three viral MPs: TGB1, TGB2 and TGB3. Particularly, TGB1 interacts with Fib [31]. BSMV TGB1 is a protein with RNA-binding, RNA helicase and ATPase activities [41–43]. The C-terminus of TGB1 binds BSMV gRNAs and sgRNAs to form RNP complexes that are thought to be involved in cell-to-cell movement [44]. Li et al. (2018) found that BSMV TGB1 localizes partially in the nucleus and nucleolus when expressed alone, or in BSMV-infected cells. TGB1 contains NLS and NoLS between 227–238 and 95–104 amino acids, respectively. TGB1 NoLS mutations reduced BSMV cell-to-cell accumulation and movement in inoculated leaves, in addition to systemic movement in *N. benthamiana* [31]. Based on the latter, the possible interaction with Fib was examined. It turned out that the BSMV TGB1 protein interacts with the *N. benthamiana* Fib 2 (NbFib2) GAR domain, while the abolition of this interaction leads to reduction of TGB1 in the nuclei and nucleoli. However, it still remains to map the region of BSMV TGB1 with Fib interaction.

In addition, Fib is induced in 60–70% in BSMV-infected plants with respect to healthy plants. The mechanism of this induction has not been yet elucidated. Furthermore, silencing of NbFib2 by RNAi reduces BSMV accumulation and its cell-to-cell movement compared to wild-type plants. Finally, it was found that NbFib2 co-localizes with BSMV TGB1 protein and vRNA near the cell wall, and in conjunction with the BSMV vRNA, indicating that NbFib2 may be part of the BSMV RNP complex (Fig. 1) [31]. This report provides the first evidence that Fib interactions during plant virus infection not only affects LDM, but that it also may be a fundamental component for cell-to-cell movement.

Poa semilatent virus – TGB1

As BSMV, *Poa semilatent virus (*PSLV) are representatives of the genus Hordeivirus characterized by rod-shaped particles and helical structures [45]. Furthermore, PSLV genome consists as well of three + ssRNAs (RNA α, RNA β and RNA γ) [45, 46]. The PSLV TGB1 contains an N-terminal extension region and a C-terminal NTPase/helicase domain (HELD).

A co-localization of PSLV TGB1 (GFP-TGB1) with Fib (mRFP-AtFib) through agroinfiltration of *N. benthamiana* epidermal cells, encourages to analyze a direct protein–protein interaction. A series of Western Blot experiments with mutants of PSLV TGB1 and AtFib2 reveals that NTD of TGB1 directly interacts with fib in vitro and that the N-terminal GAR domain of Fib is necessary for this interaction (Table 1) [27]. In addition, BiFC experiments exhibited an interaction between PSLV TGB1 and AtFib2 in both, the nucleolus and in the inclusions of different sizes in the cytoplasm [27]. Taking as a reference the involvement of Fib in GRV LDM [23, 24] and BSMV cell-to-cell movement [31], both re-localize fib to the cytoplasm and form an RNP complex. Thus, Fib might be involved in PSLV cell-to-cell movement or LDM. In fact, PSLV TGB1 has two in vitro RNA-binding activities: one is associated with the extension domain and is critical for LDM and the other is associated with the NTPase/helicase domain and is relevant for the formation of cell-to-cell movement competent RNPs [47, 48]. Nevertheless, the TGB1 NTD is involved in cell-to-cell movement as well [41]. Makarov et al. (2015) suggests that the assembled PSLV TGB1-RNA complex be considered as a non-virion transport form of hordeiviral RNA facilitating both cell-to-cell and long-distance virus transport. Ultimately, infectivity studies must be conducted in order to elucidate the precise role of Fib in PSLV infection.

Beet black scorch virus – p7a

The *Beet black scorch virus* (BBSV) is a member of the genus *Necrovirus* [49]. BBSV has a single positive-stranded RNA genome that encodes six viral proteins. Confocal microscopy analysis of *N. benthamiana* epidermal cells stained with 2-(4-Amidinophenyl)-6-indolecarbamidine (DAPI) revealed that 87% of the P7a (fused to gfp) fluorescence accumulated in the nucleus. BiFC assays showed an intense interaction between P7a and Fib (Table 1) in the nucleolus and CBs. Wang et al. (2012) found through P7a mutations that ^{9}RRERRVR^{15} of the R-rich motif controls the nucleolar targeting. When P7aR5A mutant was tested to interact with Fib by BiFC, fluorescence was observed only in the nucleolus. Also, the R-rich motif of P7a is predicted to have an RNA binding domain. Consequently, this motif might be essential for cell-to-cell movement. TGB1 of BSMV relocalizes Fib to the cytoplasm where both co-localize with its viral RNA genome to promote cell-to-cell movement [31]. According to this, P7a of BBSV might relocalize Fib to the cytoplasm and form a RNP with gRNA for cell-to-cell movement, or P7a interacts with Fib as a strategy to localize in the nucleolus and CBs to undergo a nuclear role necessary for BBSV cell-to-cell movement. Nevertheless, Fib cytoplasmic localization needs further attention to uncover which of these two scenarios is the case or not.

Fibrillarin in virus-mediated suppression of RNA silencing

Potyvirus A – Nla

Potyvirus is the largest genus of plant viruses with eight genera of viruses, all of them are plant-infecting (+) ssRNA viruses. The genomic RNA of potyvirids contain

a single ORF that codes for a major polyprotein, which is proteolytically processed by virus-encoded proteinases. The polyprotein codes for the mature viral proteins P3–6K1–CI–6K2– VPg–NIaPro–NIb–CP, which are processed by the proteinase NIaPro [50, 51].

NIa localizes primarily in the nucleus and less extensively in the cytoplasm of the infected cells [50]. NIa is partially process to produce VPg and NIaPro [52]. NIaPRO has a protease activity, responsible to processed the potyviral polyprotein and a DNAse activity proposed to degrade host DNA to regulate gene expression [50, 53]. VPg interacts not only with the majority of the potyviral proteins [54, 55], as well with host proteins: the eukaryotic initiation factor eIF4E [56], a RNA helicase-like protein from peach and *Arabidopsis* (AtRH8) which is related to eIF4E [57], Poly(A)-binding protein [58], and Fib [25]. VPg is an intrinsically disordered protein, capable of interacting with several proteins, including homodimers [59], and thus to participate in multiple processes [60–62].

As mentioned above, *Potato virus A* (PVA) NIa is mainly located in the nucleus of infected cells in systemically infected leaves of *Solanum commersonii* and *N. tabacum* (Rajamäki and Valkonen, 2009, 2003) examined by immunostaining with antibodies against VPg and NIa-Pro. The PVA NIa N-terminus contain NLSI and NLSII, located at the residues from 4 to 9 and 41 to 50, respectively, both needed for efficient nuclear and nucleolar localization [25]. Also, PVA NIa accumulates in CBs. However, the nucleoli and CBs localization turned out to be independently controlled by NLS II and NLS I, respectively.

The VPg domain of PVA NIa was found to interact with Fib, in vivo (Table 1). This interaction was subsequently studied in plant cells by BiFC [25]. Interestingly, VPg-Fib interaction occurred only in nucleoli and CBs but not in the cytoplasm (Fig. 1). In GRV where Fib is required for LDM [23, 24], and in BSMV where is part of the cell-to-cell movement [31], the interaction in the cytoplasm is key to perform both processes. Therefore, this could mean the following scenarios: (1) In PVA infection, Fib might be involved in either LDM and cell-to-cell movement or both but in a different mechanism. (2) Fib is required for PVA in a different viral process rather than viral movement. (3) The VPg-Fib interaction signal in the cytoplasm is too weak to localize. Reduction of Fib expression by 30% to 80% in leaves by virus-induced gene silencing (VIGS) affected PVA accumulation by 50% but did not prevent LDM. By contrast, in similar experiments [23, 24, 29–31], the depletion of Fib resulted in the inhibition of LDM and cell-to-cell movement. Thus, an additional role of Fib in PVA infection becomes a plausible scenario.

Plant viruses evolved to counteract the plant RNA silencing machinery through viral proteins (viral suppressors of RNA silencing: VSR) that inhibit various stages of this plant defence [63]. Most potyviruses encode two viral suppressors of RNA silencing (VSRs), HCpro and VPg [25]. HCpro is probably the most studied protein of potyviruses, particularly on its ability to suppress RNA silencing [64–66]. However, there is little research in VPg VSR activity. For instance, VPg of *Turnip mosaic virus* and other potyviruses mediate the degradation of SGS3, a key host protein, and its interacting and functional partner RDR6, both essential components of the RNA silencing pathway [67, 68].

Host genes are known to act as negative regulators of RNA silencing [63, 69, 70]. The NgRBP, a glycine-rich RNA binding protein (similar to Fib in the high content of glycine and the RNA binding property) from *N. glutinosa* suppress local and systemic RNA silencing induced by sense RNA or dsRNA. Mutational analysis of NgRBP demonstrates that the RNA motif region is necessary to maintain its RNA- silencing suppression activity. Also, NgRBP was able to interact with the 3´end GFP mRNA and dsRNA, thus it is suggested that NgRBP blocks dsRNA synthesis by RdRp at the beginning of RNA silencing, and consequently, the RISC complex formation by competitive dsRNA binding [63].

PVA VPg interacts with Fib in nucleolus and CBs, both known centers for small RNAs, including siRNAs and microRNAs that regulate gene expression posttranscriptionally [71–73]. Thus, Fib may be a vehicle for VPg to undergo its VSR activity in nucleoli and CBs. Furthermore, Fib interacts with RNAs of different lengths and types including dsRNA and viral RNA in vitro [18]. As Anandalakshmi et al. (2000) speculate with NgRBP, Fib might impede RISC complex formation through competitive dsRNA binding. Alternatively, the VPg-Fib interaction may affect host transcription or pre-mRNA processing, both processes in which Fib is involved, suggesting an explanation to the shutdown of host gene expression during potyvirus infection [74]. Further studies need to explore why Fib interacts with a VSR protein.

Cucumber mosaic virus – 2b

Another VSR protein present in CBs and nucleoli is the Cucumber Mosaic Virus (CMV)-2b [26]. Different hosts and CMV strains confirmed that the deletion of CMV 2b gene affected entirely or, to some extent the symptons. Thus indicating that CMV 2b is involved in symptom induction in the hosts rather than in LDM [75–79]. In addition, CMV 2b counteracts host basal defenses based on RNA silencing. 2b was shown to suppress silencing in a protoplast system [80]. In *A. thaliana* CMV infection, accumulation of 21-, 22- and 24-nt siRNA species were significantly reduced compared to the same strain infection lacking 2b gene [81]. Furthermore, Zhang et al. (2006) demonstrated that 2b and AGO1 proteins interacted in vitro and in vivo by transient co-expression or crosses

between *A. thaliana* expressing each protein, followed by specific immunoprecipitation assays. Also, protein 2b might suppress RNA silencing to some extent by blocking AGO1, due to the evidence that protein 2b inhibits AGO1 slicer activity in vitro [82]. In this line, 2b protein is capable of interacting with protein AGO4, mainly in the nucleus of infected cells [26]. In CMV 2b transgenic *A. thaliana* and CMV infection affected the regulation of transposons, mimicking the AGO4 phenotype. Thus, CMV 2b alters plant defense by interfering with AGO4-regulated transcriptional gene silencing [83].

CMV 2b protein colocalizes with Fib in nucleoli and associated bodies. Although, González et al. (2010) just reported a colocalization and not an interaction. PVA-NIa [25] and RSV-P2 [29], both VSRs, interact with Fib in nucleoli and CBs. This evidence, and that the 2b VSR activity relies significantly on the nucleoli [26, 83, 84] suggests a possible interaction between 2b and Fib. If this is the case, Fib might play a role in CMV 2b VSR activity, mainly due to its RNA binding ability and the pivotal movement through CBs and nucleoli [12].

Rice stripe virus – p2

RSV p2 is capable to interact with Fib in the nucleolus and CBs in *N. benthamiana* cells (Table 1) [29, 85]. Zheng et al. (2015) found that either NbFib2 or RSV p2 depletion prevents the systemic movement of RSV in *N. benthamiana* plants.

As mentioned above, p2-Fib interaction occurs in the nucleolus and CBs of *N. benthamiana* cells but not in the cytoplasm [29, 85]. GRV-ORF3 [23, 24] and satBaMV-P20 [30] export Fib from the nucleolus and CBs to the cytoplasm to form vRNPs for its LDM. The lack of a p2-Fib cytoplasmic interaction and formation of vRNPs, indicates that the mechanism of Fib for LDM in RSV infection is different from the GRV [23, 24] and satBaMV infection [30] or has another role necessary for RSV-LDM.

Another host protein (from *Oryza sativa*) interactor of RSV p2 is a homologue of *Arabidopsis* suppressor of gene silencing (AtSGS3), designated as OsSGS3 (Os12g09580). AtSGS3 is a cofactor of RDR6 and has been implicated in antiviral silencing [86–90]. YTH and BiFC experiments demonstrated that the interaction between p2 and OsSGS3 occurred in the cytoplasm and nucleus. The expression of the RSV p2 gene enhanced infectivity and pathogenicity of *Potato virus X* in *N. benthamiana*, indicated the functional role of p2 as a silencing suppressor [91]. The VSR activity of RSV p2 in the nucleus and the p2-Fib interaction in CBs and the nucleolus suggests a potential Fib's role in the suppression of RNA silencing.

Mulberry mosaic dwarf-associated virus – V2

Plant RNA viruses are not exclusive to show an interaction with Fib. The Mulberry mosaic dwarf-associated virus (MMDaV), a novel, unsigned species of the family *Geminiviridae*, a DNA-based genome virus, has been found to interact with Fib [32].

Yang et al. [92] showed that MMDaV V2 inhibited local RNA silencing and LDM of the RNA silencing signal, but not short-range spread of the GFP silencing signal in *N. benthamiana* plants expressing GFP. In addition, V2 was spotted to both subnuclear foci and the cytoplasm (in the absence of virus infection). Deletion mutagenesis of V2 identified the basic motif (61 to 76 a.a) as crucial for V2 to form subnuclear foci and for suppression of RNA silencing. Further investigation on the V2 subnuclear localization demonstrated an interaction with NbFib2 through Y2H and BiFC in plant cells (Table 1). A V2 NLS (from amino acids 61–77) mutant was assayed and did not interact with NbFib2 [32]. Interestingly, V2 could not colocalize with NbFib2 in the nucleolus in the context of MMDaV infection, whereas V2 was found in the nucleoplasm.

MMDaV encodes five ORFs (V1–V5) and two ORFs (RepA and Rep) on the virion-sense and the complementary-sense strands, respectively [93]. Different agrobacteria cultures harboring each MMDaV viral proteins were infiltrated into RFP-H2B *N. benthamiana* plants leaves together with bacterium containing the construct GFP-V2. The exclusion of V2 from the nucleolus to the nucleoplasm was only possible when V2 was coinfiltrated with RepA. The plausible interaction of V2-RepA was confirmed via Y2H and through BiFC was limited to the nucleoplasm. V2 was observed to form homodimers and RepA excluded it as well to the nucleoplasm. Furthermore, RepA mediates nucleolar exclusion of V2-NbFib2 complex verified by BiFC in RFP-H2B plant leaves [32].

Plants evolved to limit viral replication and spread of RNA- and DNA-encoded viruses by different mechanisms. RdRp and Dicer-like (DCL) proteins target RNA viruses to generate siRNAs, and then catalyse additional cleavage of viral RNA. DNA viruses also generate small RNAs that are subject to RNA-directed DNA methylation (RdDM) [94, 95]. Both, RNA- and DNA-based viruses encode silencing suppressors to limit the host plant defenses. The begomovirus *Tomato yellow leaf curl virus* (TYLCV) V2 protein suppresses the RdDM pathway through its localization to the CBs to interact with AGO4. TYLCV V2 protein colocalizes with Fib at the nucleolus and CBs [96], where V2 triggers its suppressor activity. MMDaV RepA protein excludes V2-NbFib2 complex from the nucleolus to the nucleoplasmic sites [32] where Fib as a CB marker [12] might serve as an anchor to locate V2 and exert its function. Thus, MMDaV V2 protein might suppress RdDM at the CBs

as TYCLV V2 protein. Nevertheless, it needs to elucidate whether V2-NbFib2 interaction is required for V2 to suppress RNA silencing.

Conclusions and future directions

Although the study of viral interactions with the nucleolus is an already defined phenomenon, the precise mechanisms and functional role of these interactions are far from being elucidated. Nucleolar proteomes during viral infection analysis together with bioinformatics techniques could be applied to explain the significance of viral-nucleolar molecule interactions. As well, classical molecular biology techniques such as knockdowns to disrupt these interactions, provides a strong indication of which infective cycle phase is involved.

Fib is a common target for animal and plant RNA viruses. Particularly, in plant viruses is involved in both types of movements (LDM and cell-to-cell movement) apparently by the formation of RNP particles. VSR proteins are known to interact with Fib at nucleoli and CBS, the significance of these associations with the major nucleolar protein may be complicity with the suppression of RNA silencing. As discussed above, Fib is recruited for different purposes and the mechanism varies from a given virus to another. Reports from animal viruses known to interact with Fib may provide good insights to investigate in plant viruses.

The research on these interactions also provides knowledge into novel nucleolar functions and processes and a way to define how to improve crops to be less susceptible to viral diseases.

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Dr. Enrique Castano Defined the subject rewrite and overall management of the review and defined the model.

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